Comparison of D$_2$O and ethanol dilutions in total body water measurements in humans

H.G.E. Endres$^1$, O. Grüner$^2$

$^1$ Institut für Klinische Pharmakologie, Klinik für Innere Medizin, Friedrich-Schiller-Universität Jena, Erlanger Allee 101, D-07747 Jena, Germany
$^2$ Institut für Rechtsmedizin an der Christian-Albrechts-Universität Kiel, Arnold Heller Strasse 12, D-24105 Kiel, Germany

Received: 1 July 1994 / Accepted: 27 July 1994

Abstract. Total body water was measured by ethanol dilution and D$_2$O stable isotope dilution in a group of 20 healthy volunteers (5 females and 15 males), predominantly 23- to 31-year-old students. Both indicator substances were given orally with an ethanol burden of 0.8 g/kg body weight and a D$_2$O burden of 0.1 g/kg body weight after 12-h food and fluid restriction. This first direct comparison of total body water (TBW) from ethanol and D$_2$O dilutions revealed the ethanol compartments to be smaller than those of D$_2$O. The quotient of TBW (ethanol)/TBW (D$_2$O) was 97.7%, which is the order of the quotient TBW (H$_2$O$_{18}$/TBW (D$_2$O) (=97%), well known from the literature and taken to represent relatively exactly the value of TBW overestimation (based on H/D exchange for acid protons) following D$_2$O dilution [36]. Thus the value of TBW (ethanol) is almost identical to that of H$_2$O$_{18}$/, which provides direct evidence that ethanol is distributed only in the body water.

Key words: Total body water – Dilution indicator – Ethanol – D$_2$O – Manganese powder

Our knowledge of water and electrolyte balance dates from the middle of the last century. After the unsuccessful attempts by von Bezold in 1857 [2] to determine total body water (TBW) "directly" by drying fresh carcasses, indicator substances came to be used to determine body water compartments and TBW. Important advances in the development of dilution methods include the following:

Keith, 1915 [25]: vital red for determination of plasma volume

Dawson, 1920 [7]: Evans' blue for determination of plasma volume

Crandall, 1934 [5]: thiocyanate as indicator of body water in the extracellular space

von Hevesy and Hofer, 1934 [23]: heavy water for determination of TBW

Danowski, 1944 [6]: thiourea for determination of TBW

Newman, 1944 [29]: mannitol as indicator of extracellular space

Pace, 1945 [33]: mean water content of lean body mass in mammals and humans: 73.2%

Gilman, 1946 [11]: thiosulfate as indicator of extracellular space

Pace, 1947 [34]: first use of tritiated water to determine TBW

Soberman, 1949 [39]: antipyrine for determination of TBW

Berger, 1950 [1]: N-acetyl-4-amino-antipyrine for determination of TBW

Lifson, 1955 [28]: introduction of double-labeled water D$_2$O for parallel determination of TBW and total CO$_2$ production

Grüner, 1957 [13]: ethanol for determination of TBW

Apart from the use of dilution indicators, two indirect methods of TBW determination are presently available. One is the registration of total-body $\gamma$-radiation from the naturally occurring radioactive potassium isotope $^{40}$K [44], the occurrence of which bears some relation to the volume of TBW. The other is registration of the electrolyte concentration in the body water employing computer-aided TBW calculations with TOBEC coils, a determination procedure similar to that of metal detector coils at
airports [22]. In this, the interference of body water electrolytes with an electromagnetic field is measured. To calculate TBW on the basis of these electrolytes they must bear an established relationship to the body water volume.

Of the two indicator substances listed above which are very similar to ordinary water, tritiated water (HTO) is hardly used today due to the disadvantages of β-radiant tritium (biological half-life about 10 days, physical half-life 12.3 years). In contrast, heavy water (D₂O and/or HDO in H₂O dilution) is currently preferred as a reference substance for TBW determination. This stems from its harmlessness (if only slight concentrations are administered) and its high similarity to tap water.

The preference for ethanol as a dilution indicator is based on the pharmacokinetic investigations of Widmark in 1932 [42]. As early as 1957 Grüner [13] demonstrated on the basis of these that alcohol is dissolved virtually only in water. Therefore lean persons show a clearly lower blood ethanol concentration (grams of ethanol per kilogram of whole blood) after intake of an ethanol dose related to their body weight than do obese persons because an increase in body fat (with increasing body weight) is associated with only a relatively small rise in body water. Pace and Rathbun demonstrated as early as 1945 [33] that so-called lean body mass consists of a constant mean water component of 73.2%. This corresponds to the (only theoretically achievable) maximum water content of the total body.

Previous comparative measurements of ethanol and some "TBW indicator substances" revealed that the amount of TBW obtained via the dilution of antipyrine [32] or via N-acetyl-4-amino-antipyrine [19] is always higher than that calculated via ethanol dilution. This corresponds to the well-known fact that a small part of the antipyrine and N-acetyl-4-amino-antipyrine is bound in the blood with protein. This also shows that ethanol is dissolved practically only in body water and not in body fat [14] since otherwise the ethanol TBW values would be higher than those in the real body water compartment.

Considering the importance of D₂O as a universally accepted reference substance for TBW determination, Endres [9] compared the ethanol TBW method directly with the D₂O method.

Materials and methods

Calculation bases of the ethanol TBW method

The basis of calculation, particularly the importance of referring ethanol concentrations to whole blood, plasma, or serum, has continued to be the subject of recent publications [24]. The underlying equation for all calculations was established by Widmark as early as 1932 [42]. If an amount of ethanol is taken up which results in a total blood alcohol concentration greater than 0.15% (0.15 g alcohol per kilogram whole blood), the enzyme system involved in alcohol metabolism works in the saturation range (reaction of 0th order). Using linear regression, the c₀ value (alcohol concentration at 0 min) can be determined from this part of the concentration-time curve. TBW is calculated via this value (see Fig. 1, Table 1). A water content of

<table>
<thead>
<tr>
<th>Volunteer 1</th>
<th>Ethanol concentration in per mille</th>
<th>60 min</th>
<th>80 min</th>
<th>100 min</th>
<th>120 min</th>
<th>140 min</th>
<th>160 min</th>
<th>180 min</th>
<th>200 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.32</td>
<td>1.17</td>
<td>1.08</td>
<td>1.02</td>
<td>1.03</td>
<td>0.97</td>
<td>0.94</td>
<td>0.88</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.61</td>
<td>1.41</td>
<td>1.31</td>
<td>1.26</td>
<td>1.24</td>
<td>1.15</td>
<td>1.15</td>
<td>1.05</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 1. Whole-blood and (blood) water ethanol concentrations (permille) in subject 1 (see Fig. 1)